CARDIO PROTECTIVE EFFECT OF THE LEAVES OF Artocarpus heterophyllus L. ON Daphnia magna

K.Periyanayagam*, V. Karthikeyan

Department of Pharmacognosy, College of Pharmacy, Madurai Medical College, Madurai, 625 020. Tamilnadu, India

Email: kpn1960@yahoo.com

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ABSTRACT

Objective: To prescreen the in vivo cardioprotective activity of the leaves of Artocarpus heterophyllus.L Family Moraceae using the model organism Daphnia magna along with preliminary phytochemical study and acute toxicity assessment.

Method: To evaluate the Cardioprotective effect of the ethyl acetate extract of the leaves of A.heterophyllus (EAAH) in vivo on the lactose induced arrhythmic heart of the cladocerans D.magna (Water flea) a novel model system for studying effects of agonists and toxins on cell signalling and ion channels in situ. Initially acute toxicity assessment, total phenolic content by UV spectral methods and ursolic acid content by X-ray fluorescence were determined.

Results: Normal mean heart beat of the D.magna at 20±2°C was found to be 191.4±1.4 beats/min (n=50). Arrhythmia was induced by lactose (20mM) in the bathing medium. The ethyl acetate extract of the leaves of Artocarpus heterophyllus (20, 40, 60, 80µg/ml) prevented the lactose induced arrhythmia in dose dependent manner. Previous assessment of toxicity showed LC50 5.88mg/L. Preliminary phytochemical screening of appropriate solvent extract of the leaves showed the presence of flavonoids, sterols, carbohydrates, proteins, tannins , phenolic compounds and absence of alkaloids, volatile oils, fixed oils, glycosides like anthroquinone, cardiac, cyanogenetic and isothiocyanate. Total phenolic, ursolic acid content of EAAH was 376.5mg/g, 134mg/g respectively. High percentage of calcium, potassium was found in addition to traces of magnesium, sulphur, zinc, strontium, manganese, aluminium.

Conclusion: Artocarpus heterophyllus L. (Jack fruit) has long been recognized and economically is of appreciable importance as a source of edible aggregate fruit. This study indicates that the ethyl acetate extract of the leaves of A.heterophyllus possesses potential cardio protective activity on the lactose induced arrhythmia of the Daphnia heart without any toxicity and mortality. It is assumed that this may be due to polyphenolic content, ursolic acid, trace elements like calcium, potassium, magnesium. Further investigation requires confirming this activity.

Keywords: Artocarpus heterophyllus, Moraceae, Cardioprotective, Daphnia magna, Ursolicacid

INTRODUCTION

Artocarpus genus (Family: Moraceae-mulberry family) received a great level of scientific interest as they consists of therapeutically active secondary metabolites and is economic source of food and widely used in traditional medicine. Artocarpus species are used as food and for traditional folk medicine in South-East Asia, Indonesia, Western part of Java and India. Artocarpus plants offer advantages as a profitable multipurpose crop for producing fruits and timber. Artocarpus has long been recognised and economically is of appreciate importance as a source of edible aggregate fruit; such as Artocarpus heterophyllus (Jack fruit), Artocarpus altilis(bread fruit) and Artocarpus chempeden (Chempedak) and yielding fairly good timber1. Artocarpus heterophyllus popularly known as jack fruit is one of the important and commonly found trees in the home gardens of India and Bangladesh. The term jujufruit is derived from the Portuguese word Jaca which in turn is adopted from the word “Chakka” of Malayalam - A regional Indian language2. Extracts of its plant parts have been applied in traditional medicine for the treatment of diarrhoea, diabetes, malarial fever, tapeworm infestation, and as wound healing, antisyphilic, expectorant and also to treat anaemia, asthma and dermatitis3. The present study investigate the cardiac effect of the ethyl acetate extract of the leaves of A.heterophyllus (containing polyphenols including flavonoids with triterpenoid ursolic acid) using the model organism Daphnia magna. The small fresh water crustacean D magna (0.2-3mm) was used in this experiment because of their transparent carapace, which allows for increased visibility of the internal organs and makes monitoring the heart rate of the individual easier4. Of all sequenced genomes belonging to the animal group composed of insects and crustaceans, Daphnia share more genes with humans5. It exhibits a short life span, rapid maturation and reproduction. The heart of the water flea, D magna, regulated by cholinergic neurons and may be useful as a model for the effect of drugs on cardiovascular function and unusual among crustaceans in that they possess myogenic hearts. Testing the effects of the drugs is simplified in D magna as the fleas are responsive to pharmacological agents added to the water in which they swim. The introduction of these pharmacological agents to water fleas may induce activity directly on the cardiac muscle6.

MATERIALS&METHODS

Lactose, Elendt and Biso medium, spirulina, ethylacetate. All chemicals used are 5d fine chemicals. For the determination of trace element by X-ray florescence Bruker 5-4 pioneer and CAMAG HPTLC with winCATS 1.4.3 software, densitometry TLC scanner (520nm) was used for HPTLC analysis. Laboscope model Microscope with Photomicrograph & CCTV.

Collection and authentication of the leaves of A.heterophyllus: The leaves of the healthy A.heterophyllus selected for our study was collected from Susendram, Kanyakumari (Dt), Tamilnadu. It was identified and authenticated by Prof. Dr.P. Jayaraman, Director of Plant Anatomy Research Institute, Tambaram, Chennai, Tamilnadu, India and Dr.Stephen, Taxonomist, Dept. of Botany, The American College, Madurai. A voucher specimen was deposited at the herbarium of Dept. of Pharmacognosy, Madurai Medical College, Madurai, Tamilnadu, India (PCG-276).
Preparation of extract: The leaves were dried at room temperature under shade and powdered, sieved (60mesh) and stored in a well closed container. Extracted with ethylacetate and filtered, evaporated under vacuum (Rotavapor RII, Buchi). The pale green residue obtained (EAAH) was stored in the refrigerator until further use.

Preliminary phytochemical screening: Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure [6, 7].

Determination of Total Phenolic Content: The total phenolic content of extracts was determined by Folin-Ciocalteau method [8]. The extracts were oxidized with Folin-Ciocalteau reagent, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting solution was measured at 760 nm after 20min. Using gallic acid as standard total phenolic content (standard curve was prepared using concentrations 25-50 mg/L) was expressed as mg GA equivalent/L of extract.

Elemental analysis by XRF Spectrometer: We have quantitatively determined the trace elements present in the A.heterophyllus leaves by X-Ray fluorescence spectrometer (XRF) which has the advantage generally being non-destructive, multi elemental, fast & cost effective [9, 10].

Preparation of solid sample:
Mix equal volume of powder and binder pressed up to 30 ton made into pellet. The binder must be free from contaminant element and low absorption. It must stable under vacuum and irradiation conditions.

HPTLC profile of EAAH:
Development of HPTLC fingerprint

Instrument
CAMAG TLC Scanner 3 "Scanner3-070408"/S/N 070408(1.41.21) was used for detection and CAMAG Linomat 5 sample applicator was used for the application of the track. Twin trough plate development chamber was used for development of chromatogram. Software used was Win CATS 1.4.3

Sample
The EAAH was dissolved in ethyl acetate to get a concentration of 2mg/ml and 2μl of this solution was used for taking HPTLC fingerprint.

Stationary Phase
Aluminium sheets pre-coated with silica gel Merck G F254 0.2mm layer thickness were used as the stationary phase.

Mobile phase
Toluene: Ethyl acetate: Methanol (7:2:1) was used as the mobile phase for development of chromatogram. The mobile phase was taken in a CAMAG twin trough glass chamber.

Detection wavelength
The developed plates were examined at wavelength 520nm in Densitometry TLC scanner 3. The TLC visualization, 3D display of the finger print profile and peak display at 520nm.

Culture of Daphnia magna: D.magna obtained from the local aquarium in Madurai, Tamilnadu, India. It was identified & authenticated by Prof (Major) P.Chandrasekaran, Principal, Manonmaniam Sundaranar University Constituent Model College, Vilathikulam, Nagalapuram 628 904, Toothukudi Dt,Tamil Nadu, India. (Formerly Faculty of PG and Research, Dept of Zoology and Biotechnology, Vivekananda College, Thiruvedakam West 625 217, Madurai, Tamilnadu, India. D.magna were cultured by using Elendt-Bias(M) medium [11] and maintained photoperiod ±12hr. Spirulina used as a feed in spring water aerated for 48hr to obtain O2 concentration not less than 4mg/ml. Experiment was carried at 20°C±2°C.

Toxicity assessment of A.heterophyllus leaf: 48 hr exposure of D.magna to different concentrations (1, 2, 3, 4, 5, 6 mg/L of EAAH was observed. One day old daphnids were selected for this study, since neonates may be more susceptible to toxic substance than elder one. Moreover more specificity, simplicity including easily handle in lab & less expensive. Temperature 20°C ±2°C is maintained. No food feed through the study. Test substance was added directly to the water at various concentrations. Mortality rate was observed after 24 hr and LC50 was determined [12].

in vivo Cardioprotective activities of the EAAH leaves on Daphnia magna: The heart rate of control & treated groups (Lactose and EAAH 20, 40, 60 and 80μg/ml) were monitored by transferring D.magna to depression slide slightly coated with petroleum jelly [13].

Heart beats were observed under light microscope and recorded by using Nikon coolpix camera. It was counted by image processing technique which allowed real time operations i.e. 25frames/sec.

RESULT
1. Preliminary phytochemical screening of EAAH leaves showed the presence of flavonoids, sterols, carbohydrates, proteins, tannins, phenolic compounds and absence of alkaloids, volatile & fixed oils, glycosides like anthroquinone, cardiac, cyanogenetic and isothiocyanate.
2. The total phenolic content of EAAH was found to be 37.65mg/g.
3. Trace element content by XRF analysis showed the presence of calcium (39.4%), potassium (29.6%), magnesium (2.06%), iron (0.99%), sulphur (1.83%), zinc (0.083%), strontium (0.23%), manganese (0.13%) and aluminium (0.005%).
4. HPTLC analysis of EAAH contains 13mg/g of ursolic acid (Figure 18&Z Table 1).
5. From the result of toxicity assessment of EAAH it is clearly observed that EAAH is safe and non toxic to D.magna. No significant mortality was observed up to 6mg/L. LC50 5.88mg/L(Fig 3).
6. The heartbeat of control, Lactose induced, EAAH 20, 40, 60, 80 μg/ml treated were found to be 191.4±1.4, 189.4±0.74, 191.4±1.17, 190.4±0.88, 199.4±0.74, 190.1±0.54bmp respectively (Fig 4).
Table-1: Rf value and area of separated compounds

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Figure-2: CO-HPTLC profile of EAAH showing the presence of Ursolic acid 3D display

Figure-3: LC50 OF EAAH Leaves

Figure-4: heart rate of control and different concentrations of EAAH treated on the lactose induced arrhythmic heart of D.magna
**DISCUSSION**

Antibacterial activity, anti cariogenic, anti cancer, hypoglycemic, α-amylase inhibitory, wound healing, anti asthmatic, anti spillic, vermifuge, lactogogue, analgesic, anti ulcer activities of leaves of _Artocarpus heterophyllus_ have been reported[13-22]. In recent years, phytochemical constituents of plants with varied pharmacological, physiological and biochemical activities have received attention. Plants rich in bioactive phytochemical reduce the risk of degenerative disorders such as cancer, diabetes, cardiovascular and oxidative dysfunction. A great number of species and aromatic herbs contain chemical compounds exhibiting antioxidant properties. Studies have shown that _Artocarpus heterophyllus_ contains many classes of compounds such as flavonoids, volatile acids, sterols and tannins. It was also reported that whole aqueous extract of the leaf observed to possess the highest phenolic content ([523.2mg/g]) Leaves contains prenyl flavones (artocarpine, artocarpetin, artoflavanet A, cyclohetepheryllin and artonins A and B) with antioxidant activity [2]. In our study it was observed that EAAH contains 376.5mg/g phenolic content. Most of the pharmacological effects can be explained by the phenolic compounds including flavonoids, stilbenoids, aryl benzofurans present in all parts of the plant [23]. It was reported that root of _A.heterophyllus_ contains beta sitosterol, ursolic acid, betulinic acid, cycloartenone and artoflavonan [24]. Ursolic acid and oleinolic acid are pentacyclic triterpenoids that are present in many medicinal herbs and other plants. It was reported that they are anti-inflammatory, hepatoprotective, analgesic, cardio tonic, antihyperlipidemic, sedative [25]. It prompted us to find out the presence of ursolic acid in the leaf of _A.heterophyllus_. It was found out by HPTLC that EAAH contains 134 mg/g of ursolic acid. Based on the above facts we have investigated the effect of EAAH on the lactose induced arrhythmia of _D.magna_ heart. The results clearly showed the dose dependent protective effect on lactose induced arrhythmia of the heart of _D.magna_ by EAAH (Fig.2). It is assumed that this cardiodprotective effect may be due to the phenolic content, ursolic acid and the influence of abundant calcium, potassium, magnesium content and antioxidant activity. Assessment of acute toxicity study reveals its safety and non-toxic nature. So it is concluded that leaves of _Artocarpus heterophyllus_ possesses cardio protective effect without toxicity. Further investigation on animal model and clinical trials are required.

**Conflict of statement interest**

We do not have any conflict of interest.

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